"The Assimilation of Carbon by Green Plants from certain Organic Compounds." By E. HAMILTON ACTON, M.A., Fellow of St. John's College, Cambridge. Communicated by W. T. THISELTON DYER, C.M.G., F.R.S. Received April 20,—Read May 16, 1889.

The recent synthesis of a true glucose ("acrose") by Fischer and Tafel,* from acroleïn (acrylic aldehyde) and also from glycerin,† in conjunction with the additions to our knowledge of the constitution of dextrose and lævulose by Kiliani,‡ &c., suggests fresh attention to the "aldehyde theory" regarding the synthetical formation of carbohydrate in green plants.

It is now widely believed by vegetable physiologists that a glucose is produced in the first instance from CO_2 and H_2O, but the nature of the intermediate changes is still uncertain. The experiments described in this paper were commenced to ascertain whether starch can be produced in the assimilating cells of a green plant by supplying it with acroleïn or closely related bodies, and subsequently extended to other organic compounds related to carbohydrates.

The well-known theory§ that formic aldehyde (HCOH) is first produced from CO_2 and H_2O, and then becomes polymerised to glucose, has not yet received any direct experimental proof, although it is stated by Reinke‖ that formic aldehyde has been detected in the product obtained by distilling the leaves of several plants with water.

The artificial polymerisation of formic aldehyde appears to yield a complex mixture of aldehyde and ketone alcohols, which has been variously described as methylenitan, formose, pseudo-formose, &c. Quite recently Fischer¶ and Loew** have independently stated that a small quantity of a true glucose can be proved to occur in "formose" by the phenylhydrazine reaction.†† According to Loew this polymerisation only occurs with dilute solutions of the aldehyde, and better with PbO or $Pb(OH)_2$ than $Ca(OH)_2$.

Loew supports the view that formic aldehyde is formed as an

* 'Berichte der Deutsch. Chem. Gesell.,' 1888, pp. 1088, 2566.

† *Ibid.*, p. 3384.

‡ *Ibid.*, p. 221.

§ Compare Vines, 'Physiology of Plants,' Lecture 9, Cambridge, 1886.

‖ 'Berichte der Deutsch. Chem. Gesell.,' 1881, p. 2144.

¶ *Ibid.*, 1889, p. 359.

** *Ibid.*, 1889, p. 470.

†† For an account of the views as to nature of formose, pseudoformose, methylenitan, &c., generally held before the publication of Fischer and Loew's papers referred to above, see Tollens, 'Handbuch der Kohlenhydrate;' Sec. IV, 250—252, &c. Breslau, 1888.

intermediate product in the synthesis of carbohydrate by green plants from CO_2 and H_2O, but that it becomes immediately polymerised at the moment of formation; he does not, however, adduce any new physiological experiments.

Wehmer* has shown that assimilating plant cells do not form starch from solutions of formic aldehyde or formose, and A. Meyer that this is also true for solutions of aldehyde (acetic) and trioxymethylene.

A. Meyer's paper ('Botan. Zeitung,' 1886, pp. 81, 105, 129, 145) is frequently referred to throughout the following pages.

It is well known† that starch is formed by the leaves of green plants when they are supplied with solutions of glucose or cane-sugar (saccharon): but very few experiments have been made to ascertain how far this is true for other organic compounds. A. Meyer has extended such investigations to the behaviour of leaves placed in solutions of other carbohydrates and a few other compounds; his researches are frequently referred to throughout the following pages. He found that starch is formed by leaves placed in solutions of glucose, saccharon, mannite, inulin, and glycerin.

Meyer's method consisted in placing leaves which had been deprived of starch in the dark in solutions of the substances. I have extended the investigation to other substances—especially aldehydes and substances related chemically to carbohydrates, using different methods and devoting especial attention to the formation or not of starch in the leaves of green plants when organic substances are supplied through the medium of their roots and not directly to the leaves.

E. Laurent‡ has confirmed Meyer's observation that starch is formed from glycerin.

Wehmer's negative results with formic aldehyde and formose have been already alluded to.

Meyer (*loc. cit.*) has shown that starch is not formed by leaves from solutions of raffinose, inosite, erythrite, dulcite,§ trioxymethylene, aldehyde (acetic).

Neither Meyer, Laurent, nor Wehmer describes any experiments with reference to the supply of the substances used to the roots of plants.

In the following pages where the compounds used have also been employed by Meyer, as described above, his results are stated in giving details of experiments, but I did not generally make observa-

* 'Berichte der Deutsch. Chem. Gesell.,' 1887, p. 2614.

† Meyer's paper gives full references to previous experiments on this point.

‡ 'Botan. Zeitung,' 1886, p. 751.

§ Full information concerning the relation of these bodies to the glucoses is given by Tollens ('Handbuch der Kohlenhydrate').

tions on shoots (*vide* groups A and C in detailed account) where Meyer has obtained positive results.

I am indebted to Professor S. H. Vines for the suggestion to experiment with an "extract of natural humus" (see No. 12, p. 172).

Methods and Apparatus Employed.

The experiments with each of the substances employed are classified as follows:—

A. Experiments with cut branches.

B. Experiments with solutions supplied to the roots of plants placed in a culture liquid, or in a few cases in sand moistened with the same.

C. Experiments where the solutions were applied externally by placing on the upper surfaces of leaves.

All the investigations were made as far as possible with plants, shoots, leaves, &c., in a healthy condition, and results are not stated in any cases where there was reason to believe that the plants, &c., had been injured by preliminary manipulations or exceptionally unfavourable conditions during the progress of the trials.

As is generally the case in experiments with culture solutions, algæ, fungi, &c., often developed in the solutions, although the cylinders were surrounded with black paper and the liquids had been previously boiled; where this occurred to any considerable extent the cultures were repeated with fresh plants. In a few cases the roots of the plants were placed in damp sand and the sand moistened with the substances in use. The sand had been strongly heated in a muffle just before using. The words "sand culture," placed against some of the results in the detailed account, signify that this method had been used instead of the ordinary water culture.

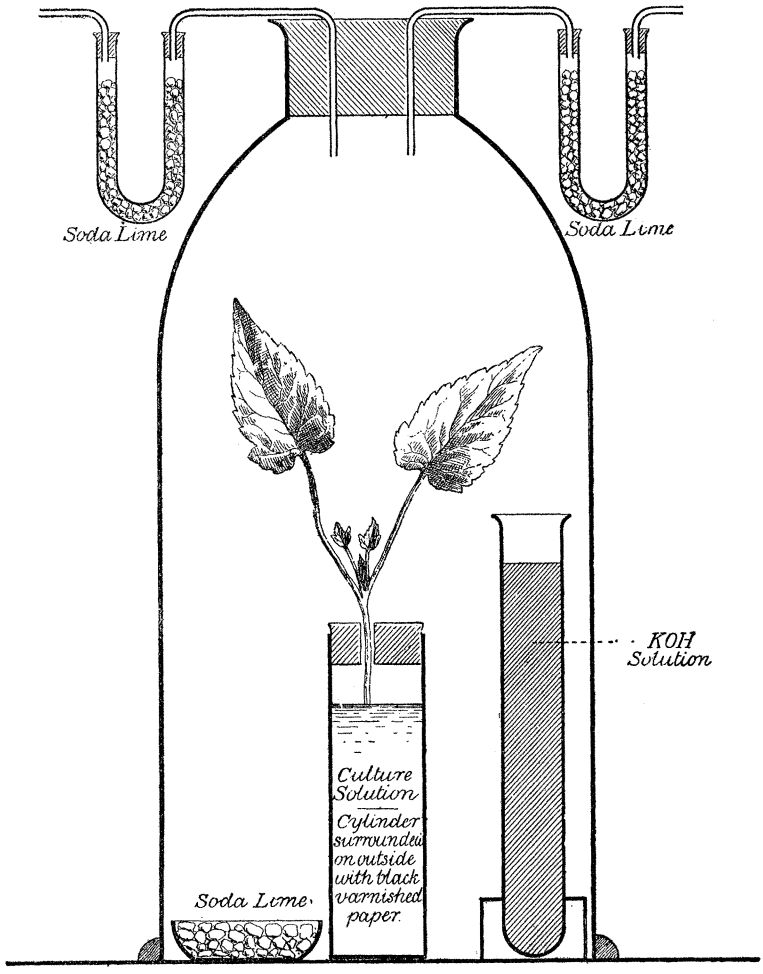
To deprive the leaves and tissues of starch at the beginning of the experiments, two methods were resorted to:—

(1.) Placing in the dark until portions of the leaves were shown by testing to be completely free from starch.

(2.) Placing under a bell-jar with substances which entirely remove all CO_2 from the air until the same result was obtained. Of these methods the latter was found to be the more convenient and used in nearly all cases. In the case of seedling plants the cotyledons must obviously be removed before placing under the bell-jar; but this operation need not cause any injury to the plant if carefully performed and the young plant has formed sufficient ordinary foliage leaves to be independent of the cotyledons. The apparatus used was as described on p. 154, and is represented in section by diagram No. 1 on the opposite page.

In this apparatus the branches, shoots, &c., freshly cut off under

DIAGRAM NO. 1.



water were placed in a cylinder containing the culture solution and the seedling plants either in the same or in a cylinder containing damp sand moistened with the culture solution. When it had been found by testing portions of the tissues that the plants, &c., were entirely free from starch, they were at once transferred to fresh cylinders containing the different solutions and placed under other bell-jars similarly fitted.

The apparatus shown in diagram No. 1 and described on the next page was always used in the first instance, but where positive results

were obtained the trials were repeated, using the modified cylinder described below and figured on p. 155 (diagram No. 2).

In testing the tissues for starch Sachs' well-known method was used; in cases where the results were negative the "potash method" recommended for small quantities of the substance was employed. I generally also made a micro-chemical examination of portions of the tissues in addition to the direct tests.

The bell-jar (see diagram No. 1) is accurately ground to fit the glass plate, the surfaces in contact are covered with a mixture of vaseline, resin, and beeswax, which is extremely tenacious, and entirely prevents any access of air in this direction.*

The india-rubber stopper is perforated with two holes, through which glass tubes are inserted connected with soda-lime U-tubes; this arrangement allows a free circulation between air in the bell-jar and external atmosphere, but entirely deprives any air entering the apparatus of CO_2. Any CO_2 derived from respiration of the plants is at once absorbed by the KOH or soda-lime, so that the air in the bell-jar is entirely destitute of CO_2 during the whole course of experiment.

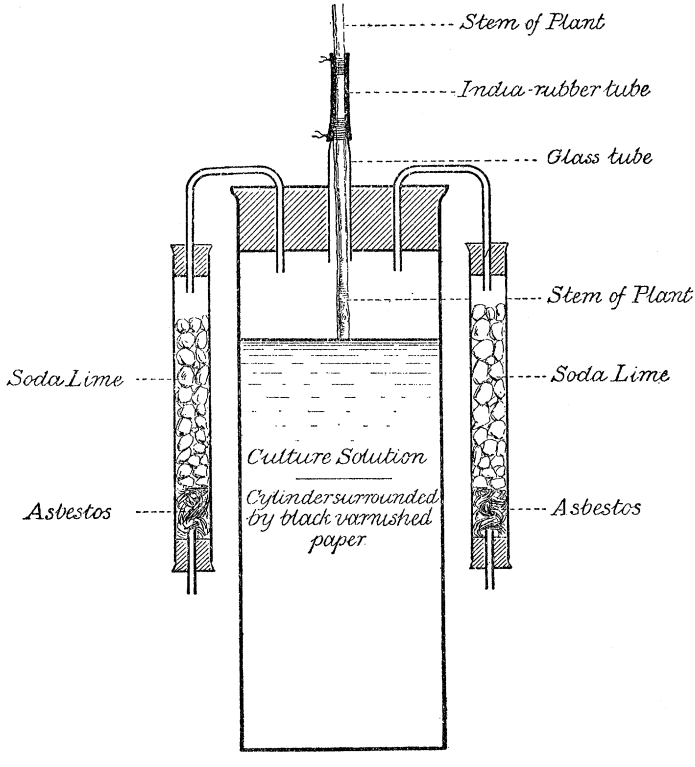
Two vessels of water (not shown in diagram No. 1) are also placed under the bell-jar to prevent any chance of the air being rendered too dry by the soda-lime. The water in these vessels is deprived of any CO_2 which it may contain in solution by the addition of baryta-water ($Ba(OH)_2$).

Since the experiments showing a positive result are open to the possible objection that CO_2 might be evolved by decomposition in the solution, and be absorbed by the leaves before it was taken up by the soda-lime or potash, I repeated these with the modified apparatus shown on the opposite page (diagram No. 2).

In this case any CO_2 evolved from decompositions in the culture solution could not find its way to the leaves; but, at the same time, a free circulation of air is allowed between the space at top of the cylinder and that in the bell-jar. Except for this modification in the cylinder containing the culture solution, the apparatus is the same as described above; instead of the plant stem being simply passed through a hole in cork of cylinder, the insertion is made gas-tight, as

* Sachs, Godlewski, &c., in similar experiments close the bottom of the bell-jar by placing it in a dish containing strong KOH solution, through which they introduce tubes (curved) to allow a free circulation of air, &c. The arrangement described in the text is equally efficacious and more convenient to employ, as the cylinder, dishes, &c., stand on a glass plate instead of in KOH solution. In the first experiment with each apparatus used, a portion of the air was withdrawn from the bell-jar, collected over mercury, and tested for CO_2 by the ordinary methods of gas analysis at various intervals; in all cases the air in the apparatus was found to be completely free from CO_2.

DIAGRAM NO. 2.



shown in diagram, by a glass tube—fitting into the cork—having a piece of india-rubber tubing slipped over its end and a portion of the stem, fastened with fine copper binding wire in each case. Communication between the air in cylinder and that of bell-jar is provided by means of the side tubes, although these prevent the exit of any CO_2 from the cylinder.

Method for Experiments with Anacharis alsinastrum *and Water Plants.**

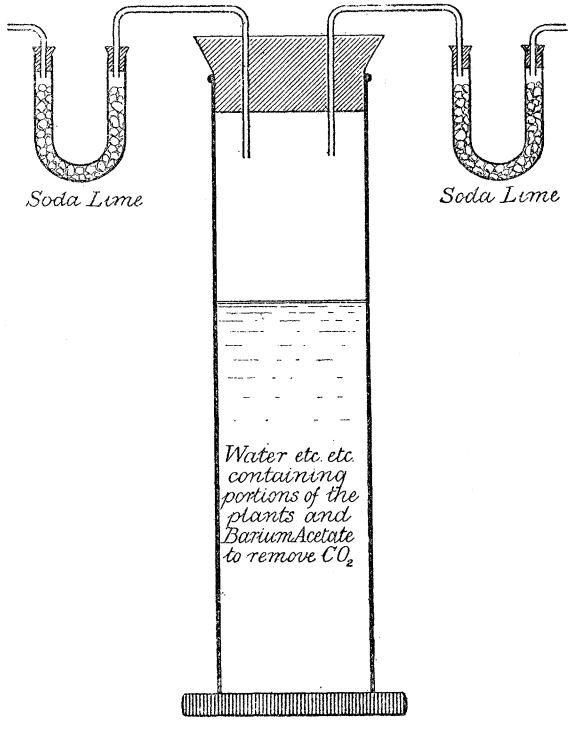
Pieces of the plant, about 8—15 cms. long, were placed in distilled water which had been deprived of any dissolved CO_2 by the addition

* In experiments with cut branches of plants I consider that the conditions are more nearly normal with water than land plants, but owing to the difficulty of keeping a supply of water plants under the requisite conditions, I have not made any very extensive use of them in these experiments.

of barium acetate solution, sufficient excess of the latter being present to withdraw from the water any CO_2 obtained from respiration. The jars containing water and plants were exposed in a window—receiving some direct sunlight—for two days, at the end of which period leaves tested as described were found to contain no traces of starch.

The plants were then rapidly transferred to other jars containing the same solutions as used in the previous experiments (see next page), with the addition of sufficient barium acetate to leave an excess of the salt for withdrawal of any CO_2 formed during the experiment, but in no case did the amount of soluble barium salts at the beginning of the experiment exceed 2·5 per cent. barium (4·2 per cent. $BaSO_4$ on precipitation). The jars in this and the previous experiment were closed by tightly fitting india-rubber corks perforated with two holes through which are inserted glass tubes connected with soda-lime U-tubes, to allow a free circulation of air in the space above the water, but to deprive any air so entering the apparatus of all traces of CO_2 (diagram No. 3).

Diagram No. 3.



The plants used for these experiments were—

Shoots (cut branches) of—

Acer pseudoplatanus, L.; *Phaseolus vulgaris*, L.; *Ranunculus acris*, L.; *Cheiranthus Cheiri*, L.; *Tilia Europœa*, L.; *Alisma plantago*, L.; *Scrophularia aquatica*, L.

Seedling plants (entire) of—

Acer pseudoplatanus, L.; *Phaseolus vulgaris*, L.; *Ph. multiflorus*, L.; *Cheiranthus Cheiri*, L.; *Quercus robur*, L.; *Campanula glomerata*, L.; *Euphorbia helioscopia*, L.; *Epilobium hirsutum*, L.

Water plants.—Shoots of—

Anacharis alsinastrum, Bab.; *Callitriche aquatica*, Sm.; *Fontinalis antipyretica*, L.; *Chara vulgaris*, L.; *Sparganium natans*, Bab.

The plants made use of in these experiments were not selected for any particular reason beyond the fact that I had an abundant supply of them at hand in the ground behind St. John's College Laboratory, where all these experiments were conducted.

The seedling plants of *Tilia*, *Acer*, *Cheiranthus*, *Campanula*, *Euphorbia* were all obtained from the place mentioned. Those of *Quercus* were brought from a neighbouring field, and planted in the garden in front of the laboratory.

The young plants of *Phaseolus multiflorus* and *P. vulgaris* were raised from seed in damp sawdust, and planted out till required for use.

The other plants were growing in the garden and immediate vicinity. As I did not in any cases find the results differing with the plants used where I considered the experiments had been satisfactorily carried out, I selected those which seemed best adapted to the apparatus in each case.

The solution used for the culture of the plants, and referred to throughout as the "culture solution," was prepared so as to contain the weights given below in 100 c.c. of the liquid.

Potassium nitrate (KNO_3).....	0·15	grams.
Magnesium chloride ($MgCl_2$).....	0·10	,,
Calcium phosphate ($Ca_3(PO_4)_2$)....	0·05	,,
Ferrous sulphate ($FeSO_4$)	0·025	,,
Calcium sulphate ($CaSO_4$).....	0·05	,,
Water (distilled).....	100	,,

In the case of the water plants there was added to the above 3—4 per cent. of barium acetate (to remove CO_2), as mentioned in describing the apparatus for water plants, which would cause some alterations in the soluble salts. Such a solution contains all the elements necessary for normal growth of a plant except carbon.

No. 1.—*Experiments with Acroleïn.*

The acroleïn was prepared by the usual method,* viz., distillation of glycerin and acid potassium sulphate, the distillate being in the first instance collected in a receiver over PbO and $CaCl_2$ to remove acrylic acid and water. The product was three times "rectified" over $CaCl_2$, and preserved in a sealed tube over a few fragments of $CaCl_2$ till required for use.

The "acroleïn-ammonia" was prepared by Claus's method,† viz., acroleïn vapour was passed into strong aqueous ammonia, the excess of ammonia driven off by warming, and the reddish solid acroleïn-ammonia precipitated by addition of excess of ether alcohol.

The solid was dissolved in water immediately before use.

Acroleïn-ammonia is a condensation product having the formula C_6H_9NO. ($2C_3H_4O + NH_3 = C_6H_9NO + H_2O$.)

The crystals of acid sodium sulphite compound, of which the crystals are somewhat insoluble, can be easily prepared in the ordinary way for these compounds. Composition, $2NaHSO_3 \cdot C_3H_4O$.

Owing to the fact that acroleïn is very liable to undergo spontaneous decomposition on standing in contact with water, and the extremely offensive nature of the substance in a free state, I employed certain soluble acroleïn compounds, as well as the uncombined aldehyde; but, although these compounds did not act so prejudicially on the plants, they caused them to assume an unhealthy appearance after 4—5 days, and two out of six plants which had been grown in the solutions under the conditions described on p. 160 failed to recover when again planted under normal circumstances, although fully supplied with water and shaded from intense direct sunlight.

Details of these experiments are given on the following pages. I think they prove conclusively that plants are unable to form starch in their leaves from acroleïn or its compounds when supplied to them as such, and that it is therefore doubtful whether the synthesis of glucose by Fischer and Tafel from acroleïn has any direct bearing on the formation of starch in plants. The results with ordinary aldehyde (acetaldehyde) and some of its compounds were also negative (see pp. 163—164), although these did not seem to have any injurious effect on the plants. In 1 per cent. solution no formation of starch could be detected, whether the substance was supplied to the roots, cut branches, or the external surface of the leaves.

No. 1.—*Experiments with Acroleïn.*

I. With free aldehyde—

* See F. Beilstein, 'Handbuch der organischen Chemie,' 2nd Edit., p. 360.

† 'Liebig's Annalen,' vol. 130, 1864, p. 185.

A. On Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 0·2 per cent. acroleïn	*Acer pseudoplatanus* *Phaseolus vulgaris* *Ranunculus acris* *Cheiranthus Cheiri*	No formation of starch (5 days). The leaves became unhealthy.
B. Solution applied to the Roots.		
Same solution.....	*Acer pseudoplatanus* (3 plants) *Phaseolus vulgaris* (4 plants)	No formation of starch (6 days).

The plants were evidently injured by this solution, presenting a yellowish appearance at the end of six days; one of the plants of *Acer* and three of the *Phaseolus* failed to recover their normal growth when planted out, and ultimately died.

C. Solution applied to Surface of Leaf.

Solution used.	Plants.	Results.
Same solution.....	*Tilia Europæa* (3 leaves) *Phaseolus vulgaris* (5 leaves)	No formation of starch (5 days). Leaves became yellow when the solution had been applied.

No. 1.—*Experiments with Acroleïn* (continued).

II. Acroleïn compounds—

(*a.*) "Acroleïn Ammonia."

A. On Cut Branches.

Solution used.	Plants.	Results.
The culture solution + ·5—1 per cent. acroleïn-ammonia	*Alisma plantago* *Tilia Europæa* *Acer pseudoplatanus* *Scrophularia aquatica*	No formation of starch (4 days). Leaves not visibly injured.
B. Solution supplied to Roots.		
Same solution as above (A)	*Cheiranthus Cheiri* *Quercus robur* *Campanula glomerata* (2 plants)	No formation of starch (6 days).

The plants were not apparently injured by this solution; on planting out they all resumed growth; the plants of *Campanula* had formed starch again after ten days (not tested earlier) after planting out.

C. Solution applied to Surface of Leaf.

Solution used.	Plants.	Results.
Same solution as above	*Acer pseudoplatanus* *Tilia Europæa*	No formation of starch.

No. 1.—*Experiments with Acroleïn* (continued).

II. Acroleïn compounds—

(*b*.) $NaHSO_3$ compound of Acroleïn.

A. On Cut Branches.

Solution used.	Plants.	Results.
The culture solution + about 2 per cent. of the crystal. $NaHSO_3$ comp.	*Phaseolus vulgaris* *Ranunculus acris* *Tilia Europæa*	Became unhealthy on 2nd day and were withered at end of 4 days. No starch.

B. Solution supplied to Roots.

Same solution as above	*Phaseolus multiflorus* *Tilia Europæa* (2 plants) *Cheiranthus Cheïri* (2 plants)	All killed at end of 5 days; showed marked injury after 48 hours. No starch.

No. 1.—*Experiments with Acroleïn* (continued).

Experiments with Water Plants.

Method and apparatus as described on pp. 155—156.

Solution used.	Plants.	Results.
The culture solution diluted with an equal vol. of distilled water + 0·25 per cent. acroleïn	*Anacharis alsinastrum* *Fontinalis antipyretica* *Chara vulgaris*	*No* starch formed. Chlorophyll markedly injured after 24 hours. No bubbles of gas evolved in bright sunlight after the first 24 hours.

Three plants of *Anacharis* from different sources were used.
Two „ *Chara* „ „ „

No. 2.—Experiments with Allyl Alcohol (C_3H_6O) ($CH_2{\cdot}CH{\cdot}CH_2OH$).

The allyl alcohol was prepared in the usual manner by distilling a mixture of glycerin and crystallised oxalic acid with a little ammonium chloride, and rectifying the crude distillate from potassium carbonate, solid potash, and finally from lime. The substance used was collected in a separate receiver (93—95° C.).

A. On Cut Branches.

Solution used.	Plant.	Results.
The culture solution + 0·5 per cent. allyl alcohol	*Tilia Europæa* *Cheiranthus Cheiri* *Ranunculus acris*	*No* starch formed. Leaves became yellow and flaccid after 6 days.
	B. Solution supplied to Roots.	
Same solution as above	*Acer pseudoplatanus* (3 plants) *Phaseolus vulgaris* (2 plants) (Sand culture). *Quercus robur* (2 plants)	*No* starch formed in leaves (6 days). Plants were decidedly injured.

On planting out after the experiment, two plants of *Acer*, both of *Phaseolus*, and one of *Quercus* failed to resume growth, and ultimately (3 weeks) died.

No. 2.—*Allyl Alcohol* (continued).

Experiments with Water Plants.

Method and apparatus described on pp. 155—156.

Solution used.	Plants.	Results.
A. The culture solution diluted with an equal volume of distilled water + 0·5 per cent. allyl alcohol	*Anacharis alsinastrum* *Chara vulgaris*	Plants injured after 12 hours, and yellow or brown after 48 hours. *No starch.*
B. The culture solution diluted with twice its volume of distilled water + 0·1 per cent. allyl alcohol.	*Callitriche aquatica*	*No* starch formed (7 days). Plant not injured to outward appearance.

Not tested whether plant formed starch again under normal conditions after experiment, as specimens used were accidentally mislaid in laboratory.

No. 3.—*Experiments with Glucose (Starch-sugar).*

Commercial "pure glucose," obtained from Messrs. Hopkin and Williams, of London, was used.

A. It is well known* that cut branches of plants, leaves, &c., form starch when supplied with solutions of glucose. I did not repeat these experiments.

B. Solutions supplied to the Roots.

(1.) Solution used.	Plants.	Results.
The culture solution + 1 per cent. of glucose	*Quercus robur* *Cheiranthus Cheiri* *Euphorbia helioscopia* *Phaseolus vulgaris* *Acer pseudoplatanus*	All contained starch at the end of 4 days.

(2.) Finding that starch was formed under these circumstances, I commenced a new series of experiments, in order to observe whether the plants were able to withdraw the whole of the glucose from solutions, and how long a time was required for the first formation of starch. As the young plants of *Cheiranthus* were growing most vigorously at this time, I used them for this purpose.

The first point was easily answered in the affirmative. Using the previously mentioned culture solution (containing 1 per cent. of glucose), such an amount was taken as to contain 3·57 grams of glucose (about 400 c.c.); six plants of *Cheiranthus Cheiri*, with their roots immersed in the solution, had completely absorbed all the glucose at the end of five or six days. In another similar experiment, I found that three plants of *Acer pseudoplatanus* absorbed 1·86 gram of glucose in eight days, but a fungus mycelium was beginning to form at the end of this time, which may have assisted in removing the glucose. It was proved that all the glucose had disappeared from the solutions by the usual methods of testing, viz., with Fehling's solution, &c., &c.

In regard to the second point, I never found any formation of starch to occur with less than ten hours' exposure to light; but it is obvious that experiments of this kind are of very little value, as it is not possible to determine to what extent the previous treatment to deprive tissues of starch has affected the normal assimilation processes.

* See Introduction.

As would be expected, those plants which had been deprived of starch by keeping in the dark were considerably longer showing starch formation in their leaves than those which had been brought into a similar condition under the bell-jars by the absence of carbon supply (CO_2), although plants as nearly as possible of the same age and size were originally selected, and in each case used for the cultures as soon as they were found to be completely free from starch.

A few of the most nearly comparable results as to the time required are given below.

Plants in Culture Solution + 1 per cent. Glucose.

Plant.	Starch first detected in leaves after the lapse of	
Cheiranthus Cheiri (deprived of starch by absence of CO_2), 5 plants, A, B, C	(A) 13 hours (continuous) (B) 11 „ „ (C) 13 „ „	from time of placing in culture solution.
Cheiranthus Cheiri (deprived of starch by placing in dark), 2 plants, C″, D	(C) 13 hours 1st day; 8 hours 2nd day (D) 13 hours 1st day; 10 hours 2nd day	
Note.—About 7 hours in dark between 1st and 2nd day.		

No. 4. *Experiments with Aldehyde* (*Acetic*).

The aldehyde was purified in the usual way by saturating an ethereal solution of commercial aldehyde with gaseous ammonia collecting the crystals, and distillation with dilute H_2SO_4.

The aldehyde ammonia used was a portion of that obtained in above; the crystals would be pure.

A. On Cut Branches.

Solution used.	Plants.	Results.
Culture solution + 0·75 per cent. aldehyde	*Ranunculus acris* *Acer pseudoplatanus* *Scrophularia aquatica* *Alisma plantago*	No starch formed (5 days); leaves unhealthy towards end of experiments.
B. Solution supplied to Roots.		
Culture solution + 0·1 per cent. aldehyde (sand culture)	*Phaseolus vulgaris* *Phaseolus multiflorus* *Euphorbia helioscopia* *Cheiranthus Cheiri* (3 plants)	*No* starch formed (6 days).

On planting out at the end of experiment the plants of *Phaseolus vulgaris* and *P. multiflorus*, with two of the plants of *Cheiranthus Cheiri*, died,* but the others, although evidently injured, did not die within three weeks.

With "Aldehyde-ammonia."†

A. On Cut Branches.

Solution used.	Plants.	Results.
Culture solution + 1 per cent. aldehyde-ammonia	*Alisma plantago* *Ranunculus acris* *Tilia Europæa* *Lilium candidum*	*No* formation of starch (4 days).
B. Supplied to Roots.		
Same solution as above	*Acer pseudoplatanus* (3 plants) *Phaseolus vulgaris* (2 plants) *Cheiranthus Cheiri* (4 plants)	*No* formation of starch (8 days).

All plants resumed growth on being planted out.

No. 5. *Experiments with Glycerin.*

Commercial "pure glycerin" was used.

A. Since A. Meyer‡ has shown that leaves supplied with glycerin do form starch, and E. Laurent§ has confirmed this observation, I did not repeat these experiments.

* Since the free aldehyde is a very volatile substance, giving off vapour at ordinary temperatures, it must be considered doubtful whether the injurious effect of the substance in the above experiment is to be attributed to action on the roots in solution, or to action of the vapour on the leaves.

A few drops of pure aldehyde allowed to evaporate under a receiver containing a plant of *Acer pseudoplatanus* quickly (24 hours) caused the death of the latter, as would be expected.

† The compound aldehyde-ammonia, = $CH_3COH{\cdot}NH_3$, is probably amidethylic alcohol, $CH_3CH{\cdot}NH_2{\cdot}OH$. The tendency of this body to undergo condensation changes with formation of basic nitrogen compounds is well known. (See 'Watts' Dict. of Chem.,' vol. 1, London, 1888; "Aldines and Aldehydines.")

‡ 'Botan. Zeitung,' 1886.

§ Laurent, in 'Botan. Zeitung,' 1886, p. 151.

B. Supplied to the Roots.

Solution used.	Plants.	Results.
The culture solution + 0·5 per cent. glycerin	*Phaseolus vulgaris* *Acer pseudoplatanus* *Quercus robur* *Campanula glomerata*	All formed starch after 5 days.

In a second series of experiments with the same solution as above I found that—

Cheiranthus Cheiri—	Had formed starch	
A. 2 plants.....	after 48 hours	From time of placing in the culture solution.
B. 1 plant	„ 56 „	
C. 3 plants.....	„ 60 „	
Acer pseudoplatanus—		
A. 1 plant	„ 68 „	
B. 3 plants.....	„ 74 „	

For experiments with *Acer pseudoplatanus*, L. in solutions with varying amounts of glycerin, I found that no starch was formed when the solution was stronger than 10 per cent. glycerin,* and solutions 15—20 per cent. glycerin decidedly injured the plant in twelve hours, and ultimately caused its death.

The same results are obtained with other plants, *e.g.*, *Quercus robur* and *Euphorbia helioscopia*, as with *Acer pseudoplatanus*, L.

No. 6. *Experiments with Lævulinic Acid.*

The acid was prepared from the lævulose obtained by Kiliani's† process from commercial inulin.

The lævulose is boiled with dilute sulphuric acid, and the zinc salt of lævulinic acid obtained from the product by the process recommended by Grote and Tollens.‡

The ethyl ethereal salt was then obtained by decomposing the alcoholic solution of zinc salt with H_2S, filtering off the ZnS, boiling to expel H_2S, saturating with HCl, and distilling in the usual way.

* As A. Meyer states that leaves form starch when placed in 10 per cent. solutions of glycerin, it must be assumed that the root tissues are affected in these experiments, or that such strong solutions are unable to travel from the root to leaves.

† See Kiliani in 'Liebig's Annalen,' vol. 205, 1880; and also in 'Berichte Deutsch. Chem. Gesell.,' 1880, p. 2426.

‡ 'Liebig's Annalen,' vol. 175, 1875, and vol. 206, 1881.

The ethyl ethereal salt was saponified, and the barium salt obtained. From the barium salt the pure acid was obtained by decomposing with dilute H_2SO_4.*

The calcium salt used in No. 6, II, A. and B. was obtained by neutralising a portion of the pure free acid with $Ca(OH)_2$.

Experiments with "Lævulinic Acid."

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 1 per cent. lævulinic acid	*Ranunculus acris* *Alisma plantago* *Scrophularia aquatica*	*No* starch formed (5 days); not apparently injured.
B. Solution supplied to the Roots.		
Same solution as above	*Phaseolus vulgaris* *Cheiranthus Cheiri* *Quercus robur* (3 plants)	*No* starch formed (8 days).
Plants all recovered normal growth on planting out, and had formed starch after 3 to 4 days.		
C. Solution placed on Leaves.		
Same solution as above	*Acer pseudoplatanus* (with roots in culture solution), 2 plants	*No* starch formed (5 days). Leaves not apparently injured where solution had been applied.

* Lævulinic acid obtained as described from lævulose or cane-sugar has been shown by Conrad ('Liebig's Annalen,' vol. 188, 1877) to be identical with β-acetyl-propionic acid (acetyl-propionic acid = $CH_3—CO—CH_2—CH_2—COOH$) obtained by the action of baryta-water on diethyl acetosuccinate. Lævulinic acid is therefore one of the "ketonic acids," which have been so largely used in recent chemical synthesis, and the non-formation of starch by the plants from this source I regard as particularly interesting.

It was my intention at the beginning of these experiments to try the calcium or magnesium salts of "aceto-acetic" acid and "acetyl-phenyl-propionic acid," as also some of the substituted "malonic ethers" of the form $R.R'.C.(CO.C_2H_5)_2$ (R.R'. = alcohol radicles), all of which are powerful reagents in organic synthesis, but, finding the results negative with lævulinic acid, I conclude that they would probably not be different with the above-mentioned bodies.

The Calcium Salt of Lævulinic acid.

A. On Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 1 per cent. calcium lævulinate	*Ranunculus acris* *Scrophularia aquatica* *Alisma plantago*	*No* starch formed (10 days).
B. Solution supplied to the Roots.		
Same solution as above	*Cheiranthus Cheiri* *Quercus robur* *Acer pseudoplatanus*	No starch formed in the leaves (8 days).

Plants apparently uninjured, resumed growth, and had formed starch again (*Acer*) after 10 days from planting out.

No. 7. *Experiments with Saccharon (Cane-sugar).*

The saccharon used was pure cane-sugar obtained from Kahlbaum, of Berlin; it gave no reduction on heating with Fehling's solution at 100° for ten minutes. Many specimens of ordinary cane-sugar contain a considerable amount of glucose, and are obviously unsuitable for such investigations.

A. As A. Meyer and E. Laurent* have shown that starch is formed by leaves, cut branches, &c., placed in the solutions of cane-sugar, I did not repeat these experiments.

B. Solution supplied to the Roots.

Solution used.	Plants.	Results.
Culture solution + 0·5 per cent. saccharon	*Acer pseudoplatanus* *Cheiranthus Cheiri* (3 plants) *Phaseolus vulgaris* (2 plants) *Euphorbia helioscopia*	Starch was formed at end of 4 days in all the leaves.

Wishing to determine whether saccharon is as readily absorbed by the roots of plants as glucose (compare No. 3, p. 162), I selected six young plants of *Cheiranthus Cheiri*, as nearly as possible of equal size, so that three of them were about the same weight as the other three (*a* = three plants, B = three nearly similar plants; B weighed 0·01 gram more than *a*); (*a*) were placed in a cylinder containing 100 c.c. of the culture solution + 0·5 glucose; B in a similar

* *Loc. cit.*

cylinder containing 100 c.c. of the culture solution + 0·5 gram saccharon.

The two cylinders were placed under the same bell-jar and arranged so as to be as nearly as possible illuminated to the same extent.

After three days (the leaves of (*a*) and (B) then containing starch) I determined the remaining glucose and saccharon in the cylinders by making up to an equal volume in each case and withdrawing an aliquot part for analysis.

Of the glucose 0·237 gram remained (absorbed 0·263 gram).

Of the saccharon 0·302 gram remained (absorbed 0·198 gram).

This experiment was confirmed by arranging another six plants in a similar manner and testing each day how much of the glucose and saccharon had been removed during the preceding twenty-four hours.

Commencing on the second day after placing the cylinders in the bell-jar, it was found at the beginning of the third day that—

(2nd to 3rd day), in 24 hours, 0·085 gram glucose, 0·061 gram saccharon.

(3rd to 4th day), in 24 hours, 0·074 gram glucose, 0·062 gram saccharon.

I therefore conclude that glucose is more readily taken up by the roots of plants (from 5 per cent. solutions) than saccharon.*

No. 8. *Experiments with "Dextrins."*

The dextrins used were of two kinds:—1. Erythro-dextrin; 2. Achroo-dextrin.

Erythro-dextrin used was obtained as "dextrin" in dry state from Messrs. Hopkin and Williams, of London. It was well washed with strong alcohol before solution in water, to remove any traces of glucose.

The achroo-dextrin was prepared from the above by heating with 5 per cent. H_2SO_4 on a "calcium chloride bath" (temperature above 100°) till the solution, neutralised with $BaCO_3$, gave no trace of reaction with I in KI.

The dextrin was then precipitated by the addition of strong (95 per cent.) alcohol and washed with the same until the washings were perfectly free from "reducing" substances, dried, and dissolved in water.

* This result is not in keeping with the experiences of A. Meyer as to the relative value of glucose and saccharon. When leaves are placed in 10 per cent. solutions he finds starch to be more readily formed from saccharon than dextrose (glucose). I have not experimented with "lævulose."

1. Erythro-dextrin.

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 1 p. c. erythro-dextrin	*Acer pseudoplatanus*	No starch formed in the leaves (5 days).
	Tilia Europæa	Same as above } no starch.
	Phaseolus multiflorus	Same as above }

2. Achroo-dextrin.

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 1·5 p. c. achroo-dextrin	Same plants as above (Erythro-dextrin)	*No* starch formed (5 days).
	B. Supplied to Roots.	
Same as 2A above ..	*Epilobium hirsutum*	*No* starch formed in the leaves (5 days).
	Tilia Europæa	
(Sand culture)	*Cheiranthus Cheiri*	*No* starch formed in leaves (5 days).

All plants resumed growth and formed starch again when planted out.

No. 9. *Experiments with Inulin.*

Commercial inulin, obtained from Messrs. Hopkin and Williams, of London, was used. It was thoroughly washed with strong alcohol (to remove any glucoses) and dried *in vacuo* before solution in water. The solutions were used immediately after preparation.*

A. A. Meyer† has shown that leaves do form starch from solutions of inulin. I did not repeat this experiment.

* This experiment with inulin I regard as inconclusive, because it is not probable that the substance used was pure. Solutions of inulin on standing for any length of time I have always found to contain lævulose, from which the starch detected might have been produced.

Kiliani ('Liebig's Annalen,' vol. 205, 1880) has pointed out the very great difficulty of obtaining pure inulin (compare also J. R. Green, 'Annals of Botany,' vol. 1, No. III).

† *Loc. cit.*

B. Solution supplied to the Roots.

Solution used.	Plants.	Results.
The culture solution + 1 per cent. inulin	*Acer pseudoplatanus* *Cheiranthus Cheiri*	Starch was formed in the leaves at the end of 5 days (not tested earlier).

No. 10. *Experiments with "Soluble Starch."*

The starch solution was prepared by pouring starch (wheat-starch) rubbed into a thin paste with water (cold) into an excess of boiling water and boiled for five minutes; on cooling the solution was filtered through paper and diluted till the strength was about 1 per cent.*

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + about 1 per cent. starch (soluble)	*Acer pseudoplatanus*	Starch grains formed in the leaves after 24 hours, abundant after 48 hours.
	Tilia Europæa	Ditto after 48 hours; not so abundant as above; increased after 3 days.

B. Supplied to Roots.

Solution used.	Plants.	Results.
(a.) Same solution as in 10 A	*Acer pseudoplatanus* *Epilobium hirsutum*† *Phaseolus multiflorus* *Tilia Europæa*	No starch formed (6 days). Ditto. Ditto.

* Sachs' method being obviously inapplicable in this case, micro-chemical observations on sections were used to detect the starch grains in the leaves.

† The two seedlings of *E. hirsutum* used were apparently injured in adding the additional soluble starch, as they withered during the second six days. As the results were negative in other cases, this experiment was not repeated.

Except in case of *E. hirsutum* (see note above), the plants all resumed growth and formed starch after several days when planted out.

(b.) More "soluble starch" added to the culture solution in each of the above till solution contained about 7 per cent. "soluble starch," and experiments continued for another six days with same plants.

Same plants. No starch formed.

No. 11. *Experiments with Glycogen.*

An ordinary solution of glycogen obtained by extracting the liver of a freshly-killed rabbit was purified by Cl. Bernard's* method, in which the glycogen is first precipitated by alcohol and the well-washed precipitate (with alcohol) dissolved in strong potash and boiled for half an hour. The solution is then diluted, filtered, and again precipitated in alcohol, well washed with the same, and the precipitate dissolved in water. The aqueous solution is strongly acidified with acetic acid (to render the insoluble (in alcohol) potassium carbonate soluble as potassium acetate), finally precipitated with alcohol, well washed with the same, and dried at a low temperature. If kept for any length of time after the preparation, the dry powder was thoroughly washed with alcohol and again dried before making the solutions used in the experiments.

The solutions remained opalescent and did not contain any "reducing substances" after the experiments; this was proved by precipitating the glycogen, &c., with strong alcohol and testing the filtrate after evaporating off the alcohol, &c., &c., in the usual way.

No. 11.—*Experiments with Glycogen.*

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 1·5 per cent. glycogen	*Alisma plantago* *Ranunculus acris* *Epilobium hirsutum*	*No* starch formed (6 days). Leaves not apparently injured.
B. Solution supplied to Roots.		
The culture solution + 1 per cent. glycogen.	*Acer pseudoplatanus* *Phaseolus vulgaris* (2 plants) *Cheiranthus Cheiri* (4 plants)	*No* starch in the leaves at end of 6 days.

The plants were not apparently injured, and resumed growth on being planted out.

* See Hoppe-Seyler, 'Traité d'Analyse Chimique appliquée à la Physiologie. Paris, 1887 (Translation from German), pp. 147—148.

No. 12. *Experiments with "Extract of Natural Humus."*

An extract of "humus" was obtained by digesting the soil—a light leaf mould—with dilute alcohol on a water-bath for eight hours and filtering through asbestos and powdered glass. The alcohol was distilled off on a water-bath, the residual solution diluted with water, and again filtered as above. 100 c.c. of this solution evaporated to dryness at 110° C. left 0·3708 grain of solid residue, which showed on combustion that it contained 15 per cent. of carbon.*

As bacteria, fungi, algæ, &c., rapidly make their appearance when such a solution is allowed to stand, I found it convenient to keep the alcoholic extract, referred to above, and evaporate off the alcohol just before use.

No. 12.—*Experiments with "Extract of Natural Humus."*

A. With Cut Branches.

Solution used.	Plants.	Results.
I. Culture solution + 20 c.c. of the "extract"	*Scrophularia aquatica* *Tilia Europæa* *Phaseolus vulgaris*	*No* formation of starch (6 days).
II. Ditto	*Cheiranthus Cheiri*	*No* starch (8 days).

B. Solution supplied to the Roots.

Same solution as above	*Acer pseudoplatanus* (2 plants) *Quercus robur* (2 plants) *Phaseolus vulgaris* (1 plant) *Cheiranthus Cheiri*	Starch *was* formed in the leaves, but in all cases only a small quantity after 6 days (not tested earlier).

* The residue contains a small quantity of nitrogen, but the amount varies greatly in different extracts. I have not experimented with any extract of "natural" humus perfectly free from N (compare Exp. No. 13).

"*Humus Extract.*"

Experiments with Water Plants.

(Method and apparatus as described on pp. 155—156).

Solution used.	Plants.	Results.
A. The culture solution diluted with twice its volume of distilled water + "humus extract"	*Sparganium natans* (parts of 4 plants) *Callitriche aquatica*	*No* starch formed. Plants not injured after 10 days. No bubbles of gas evolved in bright sunlight after six hours.
B. Some humus-containing soil which had been heated to low red-heat (10 hours) in a "muffle" and cooled in a desiccator added to above solution just before transferring the plant to solution	*Callitriche aquatica*	Ditto ditto

No. 13. *Experiments with the "Humus-like" product of Alkalies on Cane-Sugar.**

Cane-sugar (saccharon)† was boiled with strong solution of KOH for half an hour, and the whole solution diluted with water and then acidified with HCl, which causes the separation of flocculent brown "humus-like" substances. The precipitate was washed with dilute acid, dried, and then boiled with water; the aqueous extract so prepared was used in these experiments after filtering through paper.

The above aqueous extract contained about 2 per cent. of solid substances.

* The chemical nature of these brown "humus-like" substances is very little known, but they are generally assumed by chemists to be closely related to the chief constituents of natural "humus," which must be chiefly derived from the decomposition of cellulose and ligneous matter. (Compare Beilstein, 'Handb. d. Org. Chem.,' 2nd Edition.—"Huminsubstanz.")

† Compare Conrad and Guthzeit, in 'Berichte Deutsch. Chem. Gesell.,' 1885, p. 439.

No. 13.—*Experiments with the "Humus like" Product of Alkalies on Cane-Sugar.**

A. With Cut Branches.

Solution used.	Plants.	Results.
The culture solution + 25 c.c. of the aqueous extract	*Tilia Europæa* *Phaseolus vulgaris* *Euphorbia helioscopia*	*No* starch formed (6 days).
B. Solution supplied to the Roots.		
Same solution as above	*Cheiranthus Cheiri* (3 plants) *Acer pseudoplatanus* (1 plant) *Quercus robur*	*No* formation of starch in the leaves (8 days).

The plants resumed growth on being again planted out after the experiment.

Abstract of Results of Experiments.

1. Starch formed when compound is supplied either direct to leaves or to roots, with—

Glucose,
Glycerin,
Saccharon,
Inulin.

(Observed by A. Meyer for "supplied to shoots"). Experiments on shoots not repeated.

2. Starch formed when the compound is supplied direct to leaves but *not* when supplied direct to roots, with—

Soluble Starch.

3. Starch formed when the compound is supplied to the roots, but *not* when supplied direct to the leaves, with—

"*Humus Extract.*"

4. Starch *not* formed at all—with acroleïn, or compounds; allyl alcohol; dextrin; glycogen; aldehyde or compounds; lævulinic acid; artificial humus substance.

5. Glucose more readily taken up by roots from 0·5 per cent. solution than saccharon. All the glucose can be withdrawn from a 1 per cent. solution by roots if left in the solution sufficiently long.

I conclude from these experiments—

* When 5 per cent. of glucose was added to a portion of the aqueous extract, and the beaker containing this mixture exposed to light, a considerable amount of fungus mycelium (? bacteria) formed in the solution by the end of 14 days.

That green plants cannot normally obtain carbon for "assimilation" from any substances except carbohydrates or bodies closely related to them; not from aldehydes or their derivatives, and not from all carbohydrates even.

That a compound may be a source of carbon when supplied to the leaves, but not when supplied to the roots, and *vice versâ*.

That (since parasitic and saprophytic plants, and especially fungi, undoubtedly do always obtain their carbon from complex organic substances) green plants, owing to the normal process of obtaining carbon being from CO_2, have to a large extent lost the power of using such substances as a source of carbon.

That many green plants (? all) behave in the same manner towards such substances.

[Contrast fungi, which often are characterised by decomposing special substances.]

That (since neither leaves nor roots can avail themselves of carbon in the form of aldehyde or its compounds—formose, allyl alcohol, acroleïn, lævulinic acid, &c.) it is still uncertain whether or not a single substance of an aldehydic or ketonic nature is really formed by plants as an intermediate product between CO_2 and H_2O and glucose (or starch); but, if such is produced, it can only be polymerised by the plant under special conditions,* probably at the moment of formation.

* Compare Loew, 'Berichte Deutsch. Chem. Gesell.,' 1889, p. 470.

DIAGRAM NO. 1.

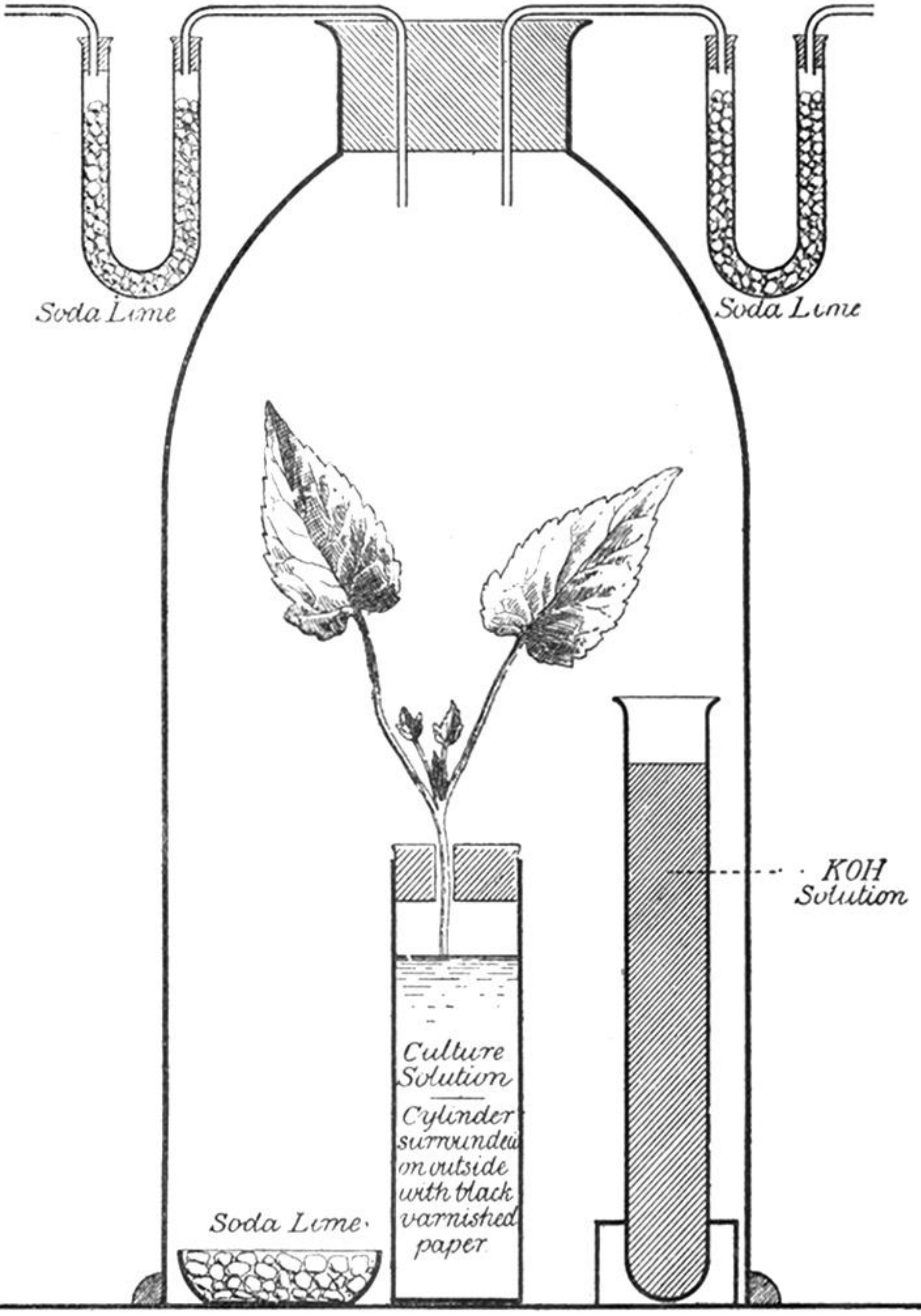

Soda Lime
Soda Lime
KOH
Solution
Culture
Solution
Cylinder
surrounded
on outside
with black
varnished
paper
Soda Lime.

DIAGRAM NO. 2.

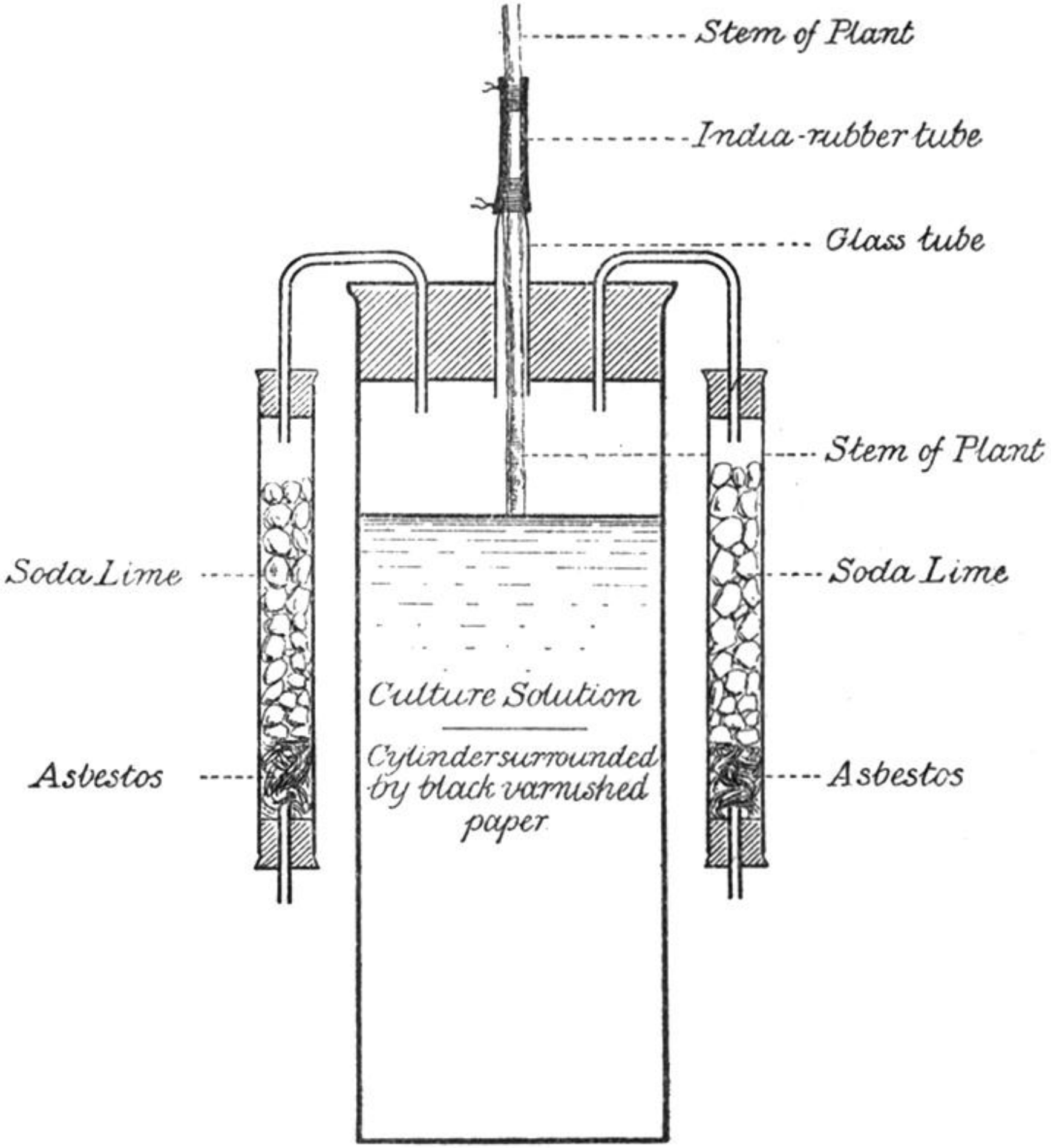
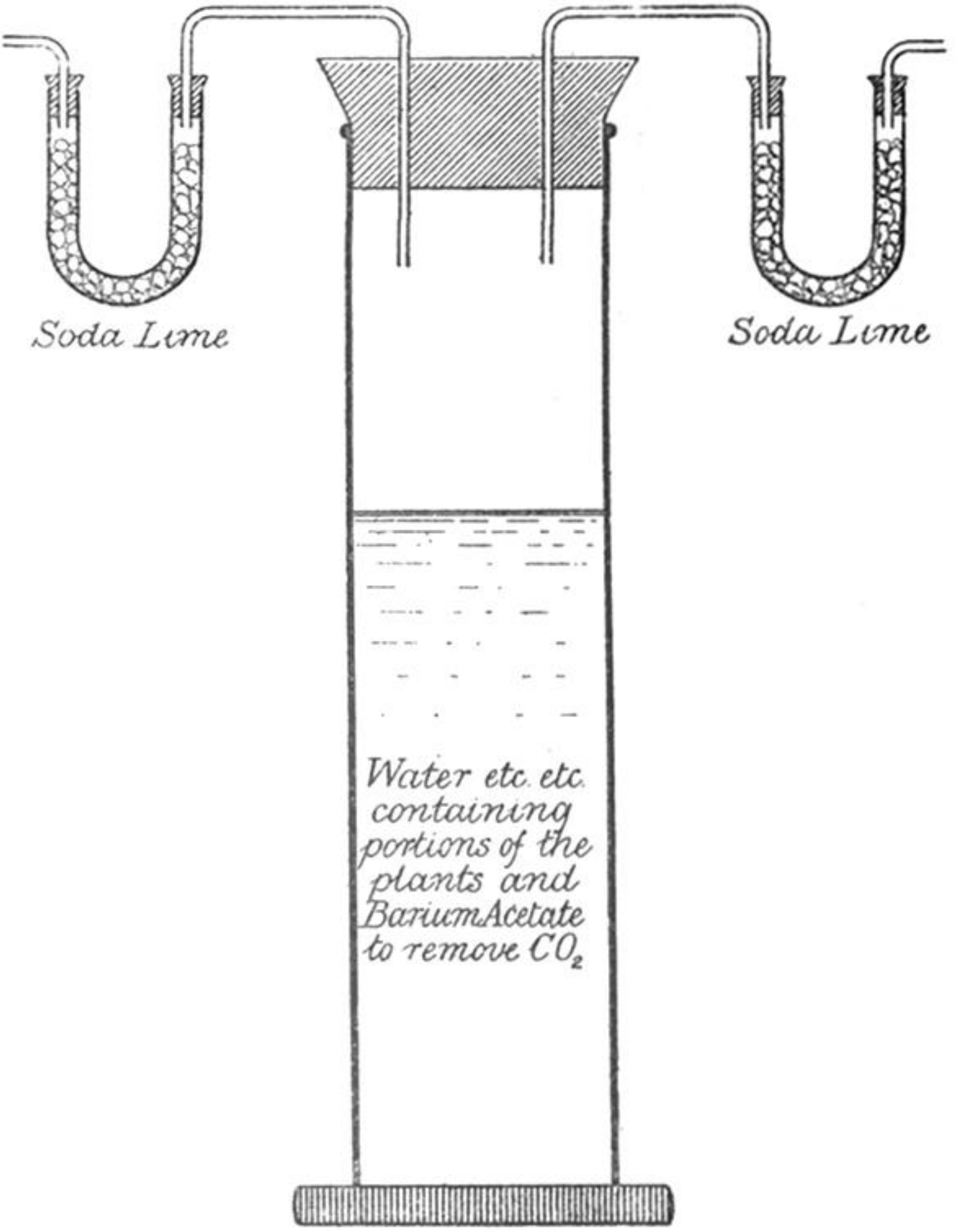

Stem of Plant
India-rubber tube
Glass tube
Stem of Plant
Soda Lime
Soda Lime
Culture Solution
Cylinder surrounded by black varnished paper
Asbestos
Asbestos

DIAGRAM NO. 3.


Soda Lime
Soda Lime
Water etc. etc. containing portions of the plants and Barium Acetate to remove CO_2